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**Authors:** Emre Fatih Ediz

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# CHEMICAL CHARACTERISTICS AND FATTY ACID COMPOSITION OF HEMP (*CANNABIS SATIVA L.*) SEED OIL: A COMPARATIVE EVALUATION OF THE NARLI VARIETY

Emre Fatih Ediz[1*]

[1]Necmettin Erbakan University, Science and Technology Research and Application Center (BİTAM), Konya, Türkiye.

[*]Corresponding Author: emrefatihediz@gmail.com
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**ABSTRACT:** This study analyzed the fatty acid profile and basic properties of the seed oil from the Narlı cultivar of *Cannabis sativa L.* farmed in Çumra Konya, Turkey. The two extraction methods evaluated were supercritical carbon-dioxide (SC-$CO_2$) extraction and mechanical cold pressing. A GC-FID system was used to measure essential analytical indices, including the distribution of fatty acids, refractive index, saponification and iodine values, along with acid and peroxide levels. The SC-$CO_2$ method yielded approximately 14% oil, whereas the cold-press method generated around 28%. In contrast to oils obtained via supercritical $CO_2$ extraction, oils from cold pressing demonstrated significantly lower oxidation values. The ratio of linoleic and α-linolenic acids was preserved. These results indicate that the extraction method significantly influences the oxidative stability and yield of Narlı hemp-seed oil. In practice, cold pressing yields an oil that is appropriate for dietary or nutritional purposes and is resistant to oxidation. Conversely, SC-$CO_2$ extraction increases the proportion of polyunsaturated fatty acids, enhancing the final oil's suitability for medicinal and cosmetic uses.

**Keywords:** *Cannabis sativa L.*, Cold press, Fatty acid composition, Hemp seed oil, Supercritical $CO_2$ extraction.

## 1. INTRODUCTION

Plant-derived bioactive substances play a essential role in traditional and modern health systems. The World Health Organization (WHO) infers that approximately 70% of the people in the world relies on herbal formulas for the treatment or prevention of disease [1]. Of the approximately seventy thousand plant species known to possess pharmacological potential, approximately twenty-one thousand are industrially processed using standard pharmaceutical methods. According to the 2013 Turkish Food Codex Regulation on Supplementary Foods, these substances are classified as natural products, extracts, or concentrates prepared in capsule, tablet, powder, or liquid forms suitable for human consumption [2].

Hemp (*Cannabis sativa L.*) is a member of the Cannabaceae family that has been cultivated for centuries as a multipurpose crop valued for its fiber, oil, food, and medicinal applications [3, 4]. Its seeds mainly consist of oil, protein, carbohydrates, and fiber accounting for approximately 25–35%, 20–25%, 20–30%, and 10–15%, respectively and also contain essential minerals such as phosphorus, magnesium, and calcium. They also provide essential vitamins such as A, C, and E, as well as β-carotene and tocopherols which enhance the nutritional and functional value of hemp derived products [5]. The seed oil mainly includes linoleic and α-linolenic acids in an approximate 3:1 proportion. This balanced composition contributes to

cardiovascular protection, anti-inflammatory effects, and cell membrane stability [6, 7]. Minor components such as phenolic compounds, lignans, and flavonoids further support its antioxidant properties [8]. In Türkiye, two industrial hemp cultivars have been officially registered: Vezir, a fiber-oriented variety, and Narlı, a seed- and oil-focused cultivar.

The oldest written record of hemp appears in the Chinese pharmacopoeia, written by the Chinese king Shen Nung Pen Ts'ao Ching in 2800 BC. This written source records its use in the treatment of fatigue, rheumatism, and malaria [9]. Archaeological evidence indicates that both Assyrian and Egyptian civilizations used hemp for therapeutic and nutritional purposes [10, 11].

The chemical composition of hemp seed oil varies significantly depending on the variety, growing environment, stage of maturity, and extraction technology [12, 13]. Traditional cold pressing which is performed at low temperatures to preserve phenolic compounds, is widely used in food production; however, the frictional heat during pressing can accelerate oxidative degradation. In Türkiye, According to the Turkish Codex "Vegetable Oils Named After the Plant" specifies that oils produced through cold pressing usually show acid values below 4.0 mg KOH $g^{-1}$ and peroxide values under 15 meq $O_2$ $kg^{-1}$. However, a few commercial products go beyond these limit [14, 15]. In recent years, supercritical carbon dioxide (SC-$CO_2$) extraction has emerged as an alternative that offers solvent-free processing, mild operating conditions, and superior retention of heat-labile components [16]. Key operational variables—pressure, temperature, and $CO_2$ flow—strongly influence both the yield and the relative abundance of extracted compounds, altering the density, viscosity, and solubility profiles of the final oil.

The study focuses on Narlı, a local hemp cultivar developed by Ondokuz Mayıs University and officially registered by the Turkish Seed Certification and Registration Center (TTSM) in 2021. Characterized by trace THC content, high seed productivity, and favorable oil yield, Narlı demonstrates consistent agronomic performance under Konya–İçeri Çumra conditions. To date, there is limited information on how extraction variables affect its physicochemical and fatty-acid characteristics. Therefore, this study provides the first comparative evaluation of cold-press and SC-$CO_2$ techniques applied to the Narlı variety, aiming to elucidate how processing parameters govern the quality and compositional profile of domestically grown hemp seed oil.

## 2. METHODS

### 2.1. Materials

Methanol (Merck, chromatography grade), n-Heptane (Merck), Diethyl ether (Chemlab), Dichloromethane (Merck, analytical grade), Chloroform (Carlo Erba), Glacial acetic acid (Merck), Acetonitrile (Sigma-Aldrich, HPLC grade), Potassium hydroxide (Afg Bioscience), Potassium iodide (Honeywell-Fluka), Sodium chloride (Sigma-Aldrich), Sodium thiosulfate (Afg Bioscience), Hydrochloric acid (Isolab), Disodium phosphate (Sigma-Aldrich), Phenolphthalein (Merck), Starch soluble (Merck), and FAME Mix Standard (Supelco 37 Component, Sigma-Aldrich) and hemp (Cannabis sativa L., Narlı variety) seeds supplied by Çumra Anadolu Production and Marketing Cooperative, harvested in October 2024.

### 2.2. Oil Extraction

#### 2.2.1. Cold Press Method

Cold pressing technique was used to obtain hemp seed oil. Seeds were refined from foreign material. Hemp seeds were kept under room temperature for 24 hours to maintain the desired moisture balance. The pressing process was carried out without any heating using a TOKUL Ekotok-1 (Türkiye) hydraulic press. The extracted oil is stored in amber bottles to avoid being affected by light and heat and stored.

### 2.2.2. Supercritical $CO_2$ Extraction

Oil was obtained from hemp seeds using supercritical carbon dioxide extraction without the use of solvents at 300 bar, 55°C and 120 minutes. The oil was stored in amber glass bottles at +4°C. Oil yield was calculated according to Equation 1;

$$Oil\ yield\ (\%): \frac{Extracted\ hemp\ oil\ (kg)}{initial\ hemp\ seed\ (kg)} * 100(\%) \quad (1)$$

### 2.3. Chemical Analyses

To determine the quality properties of the obtained hemp seed oils, FFA, PV, SV, IV, RI, and fatty acid composition analyses were performed. Each analysis was repeated three times (n = 3).

### 2.3.1. Determination of Free Fatty Acids

For this analysis, 10 g of oil was mixed with 50 mL of a petroleum ether–ethanol solution (1:1, v/v). Afterwards, 1 mL of phenolphthalein indicator was added to monitor the titration, and the mixture was titrated with 0.1 N KOH until a stable pink color was observed [17].

The percentage of free fatty acids (%FFA) was determined using Equation 2:

$$\%FFA = \frac{V \times C \times M \times 10}{m} \quad (2)$$

where $V$ is the volume of KOH used (mL), $C$ is its normality (mol/L), $M$ is the molecular weight of oleic acid (282 g/mol), and $m$ is the mass of the oil sample (g).

### 2.3.2. Determination of Peroxide Value

The peroxide value (meq $O_2$/kg) of each oil sample was determined according to the amount of active oxygen in the sample. For this procedure, 5 g of oil was blended with 30 mL of a chloroform–acetic acid mixture (3:2, v/v) and 1 mL of potassium iodide solution. The mixture was left in the dark at room temperature for 5 minutes, after which 30 mL of distilled water was added. Then, 5 mL of 1% (w/v) starch indicator was introduced, and titration was performed using 0.01 N sodium thiosulfate.

The peroxide value was calculated using Equation 3 [18]:

$$PV = \frac{V \times T \times 1000}{m} \quad (3)$$

where $V$ represents the volume of $Na_2S_2O_3$ used (mL), $T$ the normality of the titrant (N), and $m$ the weight of the oil sample (g).

### 2.3.3. Determination of Saponification Value

To ascertain the average molecular weight and triglyceride chain length of the oils, the saponification value analysis was carried out in compliance with the European Pharmacopoeia (Ph. Eur.) 2.5.6 — Determination of Saponification Value (8th Edition). For 60 minutes, around 2 g of each oil sample was refluxed with 25 mL of a Merck 0.5 N KOH solution made in 95% ethanol. Following the completion of the reaction, phenolphthalein indicator was added. After that, the mixture was titrated using a 0.5 N HCl solution. The same circumstances were used to

create and treat a blank sample, which was devoid of oil. Equation 4 was used to determine the saponification value (mg KOH/g oil) [19]:

$$\mathrm{SV} = \frac{(V_1 - V_2) \times N \times 56.1}{m} \tag{4}$$

where $V_1$ = HCl volume for the blank (mL); $V_2$ = HCl volume for the sample (mL); $N$ = HCl normality; $m$ = sample mass (g).

### 2.3.4. Determination of Iodine Value

Using the European Pharmacopoeia (Ph. Eur.) 2.5.4 — Determination of Iodine Value (8th Edition), the iodine value was calculated to assess the oils' level of unsaturation. After dissolving roughly 0.25 g of each oil sample in 15 mL of chloroform, 25 mL of Wijs solution was added. For one hour, the mixture was left in the dark. Following the reaction time, 50 mL of distilled water and 10 mL of 10% potassium iodide were added. A few drops of a starch indicator were added close to the end point of the titration, which was marked by the disappearance of the blue color. The sample was titrated using 0.1 N sodium thiosulfate solution.

The iodine value (g $I_2$/100 g oil) was calculated using the following Equation 5 [20]:

$$\mathrm{IV} = \frac{(B - S) \times N \times 12.69}{m} \tag{5}$$

where $B$ and $S$ denote the $Na_2S_2O_3$ volumes used for the blank and the sample (mL), respectively; $N$ represents the normality of the titrant; and $m$ corresponds to the oil sample mass (g).

### 2.3.5. Determination of Refractive Index

The refractive index was determined to assess the purity and degree of unsaturation of the oils in accordance with the *European Pharmacopoeia (Ph. Eur.) 2.2.6 — Determination of Refractive Index* (8th Edition) method. Measurements were carried out using a refractometer (Rudolph Research Analytical J457) operated at a constant temperature of 40 °C. Prior to analysis, the samples were equilibrated under normal circumstances and carefully placed on the prism surface to avoid moisture and air bubbles. Each sample was measured in triplicate and the mean was recorded as $n^{D40}$. Refractive index values were assessed based on the levels of unsaturated fatty acids and changes in free fatty acid content [21].

### 2.3.6. Fatty Acid Composition

A Shimadzu GC-2010 Plus gas chromatography FID system was used to assess the fatty acid methyl esters of hemp seed oils [22]. Table 1 shows the experimental conditions.

**Table 1.** GC–FID operating conditions

| Parameter | Condition |
|---|---|
| Column | 100 m × 0.25 mm × 0.20 μm silica capillary |
| Flow rate | 1.5 mL/min |

| | |
|---|---|
| Split ratio | 1:99.7 |
| Carrier gas | Dry air |

Peaks were identified by comparing their retention times with those of the 37-component FAME Mix standard. Each fatty acid's peak area in relation to the total peak area was used to determine its relative proportion.

### 2.4. Statistical Analysis

Data were analyzed statistically using SPSS v22.0. Mean differences were tested by one-way ANOVA and Duncan's multiple range test at a 95% confidence level.

## 3. RESULTS AND DISCUSSION

Hemp seed oils obtained through cold pressing and supercritical $CO_2$ extraction were analyzed for their physicochemical properties. The yield obtained by the cold pressing method was calculated as 28 ± 0.8% and the yield obtained by SC-$CO_2$ was calculated as 14 ± 0.5%. The obtained data showed results consistent with previous findings [23, 24].

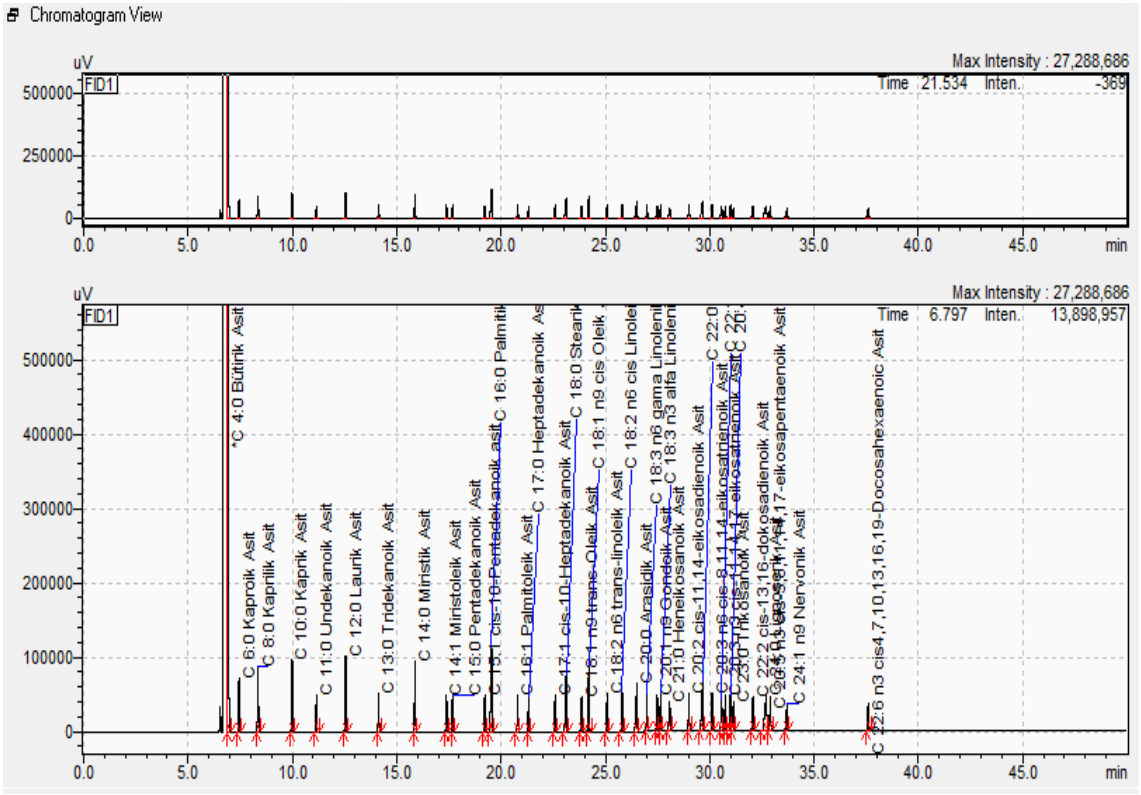


**Figure 1.** Chromatogram of the 37-component FAME Mix standard.

While the oil obtained by the cold-pressed method exhibited a light greenish-yellow hue typical of unrefined hemp seed oil, the oil obtained by the SC-$CO_2$ method was light brown-green due to the lower content of chlorophyll and carotenoid pigments removed during high-pressure extraction. Similar discoloration trends in the literature have been previously reported by Matthäus (2008) and Tura et al. (2023). These researchers stated that pigment retention was highly dependent on extraction temperature and oxygen exposure. The acid, peroxide, saponification, and iodine values of the chemical analyses of these samples are shown comparatively in Table 2 and fatty acit composition in Table 3.

**Table 2.** Physicochemical properties of hemp seed oils obtained by different extraction methods

| Parameter | Unit | Cold Press | SC-$CO_2$ |
|---|---|---|---|
| Oil yield | (%) | 28.00 ± 0.80 | 14.00 ± 0.50 |
| Acid value | (mg KOH/g) | 0.50 ± 0.03 | 8.96 ± 0.21 |
| Peroxide value | (meq $O_2$/kg) | 5.76 ± 0.18 | 8.92 ± 0.27 |
| Saponification value | (mg KOH/g) | 192.10 ± 1.90 | 189.50 ± 1.50 |
| Iodine value | (g $I_2$/100 g) | 159.08 ± 2.30 | 155.20 ± 2.10 |
| Refractive index | ($n^D4^o$) | 1.47 ± 0.00 | 1.47 ± 0.00 |

**Table 3.** Fatty acid composition

| Fatty acid | Unit | Cold Press | SC-CO2 |
|---|---|---|---|
| Palmitic acid | (C16:0) | 7.98 ± 0.14 | 8.41 ± 0.17 |
| Stearic acid | (C18:0) | 3.42 ± 0.09 | 3.89± 0.11 |
| Oleic acid | (C18:1) | 10.73 ± 0.22 | 8.44± 0.19 |
| Linoleic acid | (C18:2, ω-6) | 54.38 ± 0.68 | 65.78± 0.74 |
| α-Linolenic acid | (C18:3, ω-3) | 15.94 ± 0.33 | 15.94± 0.29 |
| Total unsaturated | FA | 81.05 ± 0.74 | 83.16± 0.79 |
| ω-6/ω-3 | ratio | 3.46:1 ± 0.09 : 1 | 3.46:1± 0.08 : 1 |

The difference in extraction methods significantly affected the composition and quality of hemp seed oil. And the oil extracted by the cold pressed method exhibited lower acid and peroxide values and higher oxidative stability. The oil obtained by the SC-$CO_2$ method, on the other hand, exhibited a more unsaturated fatty acid profile. The increase in linoleic acid is presumed to be due to the selective solubilizing power of $CO_2$ under supercritical conditions [25]. This selective extraction behavior increases the proportion of polyunsaturated fatty acids [26]. Consequently, the oil obtained by the cold-pressed method, with its lower oxidation parameters, is considered more suitable for food and nutraceutical applications, while the oil obtained by the SC-$CO_2$ method, with its higher PUFA content, is considered more suitable for cosmetic and pharmaceutical applications. Furthermore, the ratio of ω-6/ω-3 indicates that the hemp seed oils obtained by both methods maintain their nutritional balance.

## 4. CONCLUSIONS

The results obtained indicate that extraction techniques play a significant role in determining the yield and quality of the oil. The acid and peroxide values of the oil obtained by the cold-press method were 0.50 mg KOH $g^{-1}$ and 5.76 meq $O_2$ $kg^{-1}$. These results indicate that the oil obtained by the cold-press method is more oxidatively stable. Analysis of the oil obtained by supercritical $CO_2$ extraction revealed an oil richer in polyunsaturated and monounsaturated fatty acids, particularly linoleic acid (65.78%). The omega 6–3 ratio in the oils was approximately 3.46:1 by both extraction methods was determined to be within the recommended limits for the food sector. Narlı hemp seed oil, cultivated in the Konya-Içeriçumra region, is considered a valuable local resource with food applications.

## 5. FUTURE PERSPECTIVES

In future studies, efficiency and optimization studies will be conducted for SC-$CO_2$ extraction at different pressures, temperatures, and $CO_2$ flow rates.
Secondary oxidation indices ($K_{232}$, $K_{268}$, p-anisidine value) are planned.
Lovibond analysis will be carried out to assess the color properties of samples from different extraction processes.

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